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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



**WO 2004/040982 A1**

(54) Title: INHIBITING DEPOSIT FORMATION BY BACTERIA ON SURFACES OF PAPER AND BOARD MACHINES

(57) Abstract: The invention relates to a method of inhibiting the deposit formation caused by thermophilic adhering bacteria, *Deinococcus geothermali* in particular, on surfaces of paper or board machines, or removing such deposits from the said surfaces so that a concentration of at least one plant extract prepared from wood, bushes or woody, shrubby plants, or at least one plant-based substance, such as betulin or a flavonol or a mixture thereof, is added to the circulation waters of paper or board machines, the concentration being effective against thermophilic adhering bacteria.

## Inhibiting deposit formation by bacteria on surfaces of paper and board machines

The invention relates to a method of inhibiting the deposit formation by thermophilic bacteria on surfaces of paper or board machines or removing such deposits from the said surfaces.

### Field of the invention and prior art

To ensure a smooth production of paper and board machines, special chemicals, anti-microbial agents, are used to inhibit the growth of detrimental microbes in the process waters and on the surfaces thereof. These should be approved in advance. The deposits, which detach from the surfaces and cause breaks and idle time, contain detrimental bacteria as well as other bacteria, fibres, fibrils, pitch, pigments and other raw materials of papermaking. Such deposits formed by bacteria are also called biofilms. According to the newest studies (M. Kolari, J. Nuutinen and M.S. Salkinoja-Salonen, Mechanism of biofilm formation in paper machine by *Bacillus* species: the role of *Deinococcus geothermalis*, Journal of Industrial Microbiology & Biotechnology (2001) 27, pp. 343-351), one vital factor in the deposit formation is a so-called primary adhering bacterium (*Deinococcus geothermalis*), which can start the deposit formation. This bacterium is fairly common in paper machines. The purpose of preventing this bacterium from adhering to steel surfaces is thus to decrease the deposit formation that is harmful to the runnability of paper machines.

The patent publication US 6 267 897 B discloses a method of preventing biofilm formation in commercial and industrial water systems by adding an essential oil into the system. As examples of water systems, the publication cites, among others, cooling water, water in the food industry, systems of pulp and paper mills, pasteurizing apparatuses of breweries, fresh-water systems, etc. This patent publication describes a test, which studies the biofilm formation of *Sphaerotilus natans*, a slime-forming microorganism, which commonly occurs in paper mills, on glass surfaces. The results indicate that eucalyptus oil, cinnamon oil, and tea tree oil prevent the attachment of the studied bacterium on glass surfaces more effectively than the copolymer of ethylene oxide and propylene oxide that was used as a reference compound. According to this patent publication, eucalyptus oil and cinnamon oil, which are commercial drug preparations, are particularly advantageous essential oils and prepared by distillation in steam, as is well known. The other essential oils specified in this patent publication are made by distillation in steam or by compression.

The *S. natans* bacterium is not found in modern hot paper machines, because it cannot grow at temperatures of 50 to 60°C. Instead, a truly problematic bacterium in the paper machines of today is the *Deinococcus geothermalis*, which grows at high temperatures (56 to 57°C at a maximum). On the basis of the studies conducted, it  
5 can be established that the essential oils were actually not at all capable of preventing the deposit formation by the problematic bacterium *Deinococcus geothermalis*, (see the Reference Example 2 presented hereinafter).

The present invention is particularly intended to preventing such deposit formation, wherein an essential part comprises the really problematic bacterium *D.*  
10 *geothermalis* that grows at the high temperatures (about 50 to 60°C) of today's paper machines.

Accordingly, the purpose of the invention is to provide a method and an agent used therein, which can be used to effectively prevent the deposit formation caused by thermophilic bacteria, the *Deinococcus geothermalis* bacterium in particular, on  
15 surfaces of paper or board machines.

#### Description of the invention

It has now been discovered that by using natural plant extracts and certain plant-based substances (betulin and flavonols in particular), the formation of detrimental deposits in paper and board machines can be prevented by interfering with the  
20 adhesion of the actual problematic bacteria on the surfaces of the tube systems and the wet end. By using these substances, breaks and idle time can be reduced, which are caused by deposits that form on the surfaces of paper and board machines and detach from them, if the formation thereof is not inhibited. According to the laboratory research conducted, the plant extracts effectively (91 to 99.7%)  
25 prevented the adhesion of a significant factor of the deposits, namely the *Deinococcus geothermalis* bacterium, on the paper machine steal surfaces (AISI 316). The plant-based substances studied (betulin and flavonols) variably prevented (84 to 99.95%) the adhesion of the said deinococcus to the steal, depending on the substance.

30 Thus, the invention, provides a method of inhibiting the deposit formation caused by thermophilic adhering bacteria on surfaces of paper or board machines, or removing such deposits from the said surfaces, which method is characterized in that a concentration of at least one plant extract or at least one plant-based substance or a mixture thereof is added to the circulation waters of paper or board machines,  
35 the concentration being effective against thermophilic adhering bacteria.

The said plant extract may originate from trees, bushes or woody, shrubby plants, particularly from one of the following plants or parts of plants: deciduous tree, bark of a conifer, decayed conifer or a woody, shrubby plant.

Examples of suitable deciduous trees include rowan tree, birch, maple and willow.

- 5 Examples of suitable woody, shrubby plants include lingonberry and blueberry. The said bark of a conifer is preferably pine bark and the said decayed conifer is preferably decayed spruce branches.

- 10 The said plant extract can be obtained by extracting the plant or part of it with a suitable solvent or a mixture of solvents. One preferable solvent is ethanol or acetone or a mixture thereof. When using the mixture of ethanol and acetone, the volumetric ratio of ethanol to acetone may be in a range of 99:1 – 1:99, preferably within 95:5 – 5:95. Other solvents suitable for the extraction include methanol, hexane and chloroform. The mixtures of the solvents presented herein can also be used.

- 15 In this connection, the plant-based substance refers to a natural substance isolated from a plant, or a synthetic equivalent or derivative thereof. This substance as well as the said plant extract should have the ability to prevent the deposit-formation by thermophilic bacteria on surfaces and/or the ability to remove such deposits from the surfaces.

- 20 The said plant-based substance is preferably betulin or a flavonol, such as pentahydroxy flavone or trihydroxy flavone or a mixture thereof. Betulin is found in birch bark and flavonols can be obtained from the blossoms of the plants. These substances can also be prepared synthetically.

- 25 The plant extract or the plant-based substance or a mixture thereof is added to the circulation water of paper or board machines so as to obtain a product concentration, which may be 1 to 1000 ppm, preferably 10 to 500 ppm, and especially preferably 20 to 250 ppm as calculated from the dry weight of the plant extract or the plant-based substance.

- 30 The plant extract or the plant-based substance or the mixture thereof can be dosed into the circulation water of the paper or board machines either 2 to 8 times a day in cycles, or as one single dose per day.

- The plant extract of the plant-based substance or a mixture thereof can also be dosed into a container in large doses of 500 to 5000 ppm as calculated from the dry weight of the plant extract or the plant-based substance, so as to detach the adhering  
35 bacteria from the container surfaces by means of so-called shock dosing.

According to the invention, the raw extracts obtained from the said plants, or more effective components isolated from these raw extracts can be used.

The invention also relates to the use of the said plant extract or the plant-based substance, which preferably is betulin or a flavonol, or a mixture thereof for  
5 preventing the deposit formation caused by thermophilic adhering bacteria on surfaces of paper or board machines, or to remove such deposits from the said surfaces.

In the following, the invention is described in detail with the aid of laboratory studies and examples. The percentages presented in this specification are percents  
10 by weight and those of the solvents are percents by volume.

#### The studies conducted

Raw extracts of rowan tree, birch, willow, maple and spruce were prepared from chopped branches (the order of size 0.5 – 2 cm). Bark of a conifer was ground so as to obtain a similar size before extraction. The parts of both lingonberry and  
15 blueberry above ground were taken to the extraction. The extractions were carried out with a mixture of ethanol of about 90% and acetone (about 3%) (Etax B 7 solvent) for about 2 weeks at room temperature. The extracts were concentrated to a dry content of 11 to 30%. The plant extract contents mentioned in the examples refer to the content of raw extract as dry matter.

20 The adhering bacterium *D. geothermalis*, isolated from a paper machine, was kept frozen at a temperature of -70°C. For the deposit inhibition studies, it was rejuvenated in TH nutrient liquor with agitation at 45°C. The composition of the TH liquor was as follows:

	Tryptone	5 g/l
25	Yeast extract	2.5 g/l
	Water	1000 ml
	pH	6.8 – 7.0

A total of three inoculations (á 40 ml/24 h) of the rejuvenated deinococcus were  
30 added into steel biofilm devices having a liquid volume of about 4 litres. Such biofilm devices are described in the patent publication FI 95597. The initial content of deinococcus in the circulation water of the biofilm devices was  $1 \times 10^8$ - $5 \times 10^8$  pieces/ml. As circulation liquid, 0.1-percent sterilized paper machine white water was used (thin nutrient liquor in testing the pentahydroxy flavone). It was circulated  
35 in the steel (AISI 316) biofilm devices past the sample plates (7.5 x 1.5 cm) by

means of pumps at a speed of about 60 cm/s. The temperature was about 45°C. The value of acidity was kept in a pH area of 6 – 7.5. 8 similar biofilm devices were used, one of which being a control device (deinococcus only) and the other 7 devices were used to test the efficiency of the plant extracts and the plant-based substances (deinococcus + the substance to be tested). A total of 3 dosages of the substances to be tested were added (30 to 500 ppm/24 h as dry matter). The testing time was 3 days. The content of deinococcus was determined from the steel plates by means of a cultivation method. The loose deposits of the steel plates that were taken from the biofilm devices were first washed off for 2 to 3 seconds with sterile spray water (3 bar). After this, the actual deposits were detached into sterile water by means of sterile toothbrushes. The deinococcus was determined from the suspensions thus obtained by cultivation by means of a biological membrane method (0.2  $\mu$ m, Sartorius) with PCA agar, incubating for 3 days at 45°C. The composition of the PCA agar was as follows:

15	Tryptone	5 g/l
	Yeast extract	2.5 g/l
	Glucose	1 g/l
	Agar	15 g/l
	Water	1000 ml
20	pH	7.0

The deinococcus content of the steel plate of the control device was  $10^5$ - $10^7$  pieces/cm<sup>2</sup>. The effect of the plant extract and the plant-based substances was obtained by comparing the deinococcus content of the control steel plate (pieces/cm<sup>2</sup>) to that of the steel plate of the biofilm device containing the plant extract or the plant-based substance (pieces/cm<sup>2</sup>). This difference is indicated as percents in the following examples.

### Examples

#### Example 1

30 The effect of the plant extract (13% of dry matter) prepared from rowan on the inhibition of deinococcus adhesion was studied in a laboratory by means of steel biofilm devices in accordance with the method described above. The results were as follows:

Plant extract and dosage	Content of deinococcus on steel plate, pieces/cm <sup>2</sup>	Inhibition of deinococcus adhesion %
Control	$1.4 \times 10^6$	-
Rowan extract 100 ppm	$1.9 \times 10^5$	86
Rowan extract 250 ppm	$4.4 \times 10^3$	99.7

- As the results show, the rowan extract in a content of 100 to 250 ppm was fairly effective in inhibiting the adhesion of deinococcus to steel; particularly, if taking into account the high content of the deinococci, which was to be inhibited, in the circulation water of the biofilm devices ( $1 \times 10^8$ - $5 \times 10^8$  pieces/ml) and the non-frequent dosing of the substances to be tested (1 dosage/day).

### Example 2

- The effect of the other plant extracts was studied in quite a similar way than that of the rowan extract in the previous Example 1. The results were as follows:

Plant extract	Dry content of plant extract %	Addition of plant extract ppm	Inhibition of deinococcus adhesion %
Birch <sup>1</sup>	30	50	97.3
"		100	96.7
"		150	99.1
Spruce branches <sup>2</sup>	22	100	98.7
Lingonberry	26	250	94.8
Maple	13	100	92
"		250	97.2
Pine bark	25	50	97.6
Willow	11	100	91.1
"		250	98.2
"		500	98.1
Blueberry	14	500	95

<sup>1</sup> from dried birch branches

<sup>2</sup> from spruce branches decayed for about 10 years

- The results indicate that all the plant extracts in Example 2 inhibited the adhesion of deinococcus to steel, when in sufficient concentrations. The most effective ones were the extracts prepared from birch and the bark of pine, which inhibited the

adhesion of deinococcus by about 97% in a content of 50 ppm (as dry matter). The amount of the blueberry extract needed for obtaining a corresponding result was 500 ppm.

### Example 3

- 5 The ability of the plant-based substances (betulin, pentahydroxy flavone and trihydroxy flavone) to inhibit the adhesion of deinococcus to paper machine steel was studied in the same way as the ability of the plant extracts in Examples 1-2. The results were as follows:

Plant-based substance	Addition ppm	Inhibition of deinococcus adhesion %
Betulin <sup>1</sup>	50	93
Pentahydroxy flavone	30	99.95
Trihydroxy flavone	100	84

<sup>1</sup> dissolved in acetic acid

- 10 Of the plant-based substances studied, the pentahydroxy flavone was thus particularly effective in inhibiting the adhesion of deinococcus to steel.

### Reference example 1

- For the sake of comparison, the ability of the anti-microbial agent Fennosan M9 (methylene bithiocyanate as the active ingredient) to inhibit the adhesion of  
 15 deinococcus to steel surfaces was studied. The study was conducted in a similar way as in the above Examples 1 and 2. The results were as follows:

Fennosan M9 ppm	Inhibition of deinococcus adhesion %
20	70
60	99
20 100	99

- According to these results, the amount of the Fennosan M9 product needed was 60 to 100 ppm for effectively preventing the adhesion of deinococcus to steel surfaces. On the basis of the results, it can be concluded that, by means of the best plant extracts, at least as good an inhibition of deinococcus adhesion to steel can be  
 25 achieved as with that of the anti-microbial agent commonly used in paper machines, namely the methylene bithiocyanate.



**Reference example 2**

The effect of eucalyptus oil on the adhesion of the deinococcus bacterium to steel surfaces presented in the patent publication US 6 267 897 B was also studied. The study was conducted in the same way as in the above Examples 1 and 2. The results

5 were as follows:

Content of eucalyptus oil ppm	Inhibition of deinococcus adhesion %
100	0
250	37

10 On the basis of these results, we can conclude that the eucalyptus oil in 100 ppm or 250 ppm had practically no inhibitive effect. In practice, namely an effect of less than about 90% cannot be considered significant. This leads to the conclusion that eucalyptus oil has no effect on the actual adhering bacteria of hot paper machines.

15 By using, in the paper industry, the plant extracts and the plant-based substances according to the invention, it is possible to accomplish distinct advantages over the present anti-microbial agents: in utilization contents, they may be non-toxic or less toxic for the paper machine bacteria than biocides containing synthetic active ingredients. It is probable that in nature, any plant compounds that are bio-chemically produced degrade more easily and perhaps quicker into organic  
20 compounds than compounds, which are made by means of a chemical synthesis and which generally are foreign to the nature. The raw materials of the plant extracts are easy to obtain during the growth period. In a way, the plant extracts are substances of the paper machine's "own", as some of them can actually be prepared from the same wood as paper and board, even though from different parts of the wood and by  
25 different methods.

**Claims:**

1. A method of inhibiting the deposit formation caused by thermophilic adhering bacteria on surfaces of paper or board machines, or removing such deposits from  
5 the said surfaces, **characterized** in that a concentration of at least one plant extract or at least one plant-based substance or a mixture thereof is added to the circulation waters of paper or board machines, the concentration being effective against thermophilic adhering bacteria.
2. A method according to Claim 1, **characterized** in that the plant extract  
10 originates from any of the following plants or parts of plants: deciduous tree, bark of a conifer, decayed conifer or a woody, shrubby plant.
3. A method according to Claim 1 or 2, **characterized** in that the plant extract originates from any of the following plants or parts of plants: rowan tree, birch, maple, willow, lingonberry, blueberry, bark of pine or decayed spruce branches.
- 15 4. A method according to any of the preceding claims, **characterized** in that the said plant extract is obtained by extracting the plant or a part of the plant with ethanol or acetone or a mixture thereof.
5. A method according to Claim 1, **characterized** in that the plant-based  
20 substance is a natural substance isolated from a plant, or a synthetic equivalent or derivative thereof.
6. A method according to Claim 5, **characterized** in that the plant-based substance is betulin or a flavonol, such as pentahydroxy flavone or trihydroxy flavone.
7. A method according to any of the preceding claims, **characterized** in that the  
25 plant extract or the plant-based substance or a mixture thereof is added to the circulation waters of paper or board machines so as to obtain a product concentration of 1 to 1000 ppm, preferably 10 to 500 ppm, as calculated from the dry weight of the plant extract or the plant-based substance.
8. A method according to any of the preceding claims, **characterized** in that the  
30 plant extract or the plant-based substance or a mixture thereof is added to the circulation waters of paper or board machines either 2 to 8 times a day in cycles or in one single dose per day.
9. A method according to any of the preceding claims, **characterized** in that the plant extract or the plant-based substance or a mixture thereof is dosed in single

doses of 500 to 5000 ppm, as calculated from the dry weight of the plant extract or the plant-based substance, into a container for removing adhering bacteria from its surfaces by means of a so-called shock dosage.

10. A method according to any of the preceding claims, **characterized** in that the  
5 thermophilic adhering bacterium is *Deinococcus geothermalis*.

11. The use of a plant extract or a plant-based substance or a mixture thereof for inhibiting the deposit formation caused by thermophilic bacteria on surfaces of paper or board machines or for removing such deposits from the said surfaces.

12. The use according to Claim 11, **characterized** in that the said plant extract or  
10 the said plant-based substance or a mixture thereof is added to the circulation waters of paper or board machines so as to obtain a product concentration of 1 to 1000 ppm, preferably 10 to 500 ppm, as calculated from the dry weight of the plant extract or the plant-based substance.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 2003/000833

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A01N 65/00, C02F 1/50, D21H 21/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A01N, D21H, C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, CHEM. ABS DATA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002051819 A1 (CARLA H. KUHNER ET AL), 2 May 2002 (02.05.2002) --	1-12
X	WO 02078450 A1 (HERCULES INCORPORATED), 10 October 2002 (10.10.2002) --	1-12
X	US 6267897 B1 (LINDA R. ROBERTSON ET AL), 31 July 2001 (31.07.2001) --	1-12

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 16 February 2004	Date of mailing of the international search report 17 -02- 2004
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Solveig Gustavsson/Eö Telephone No. +46 8 782 25 00

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 2003/000833

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Ethnopharmacology, Volume 52, 1996, E.M. Ritch-Krc et al: "Carrier herbal medicine: an evaluation of the antimicrobial and anticancer activity in some frequently used remedies", page 151 - page 156 --	1-12
X	WO 0059834 A1 (BETZDEARBORN INC.), 12 October 2000 (12.10.2000) --	1-12
X	WO 0026466 A1 (BIOCONSULT GESELLSCHAFT FÜR BIOTECHNOLOGIE MBH), 11 May 2000 (11.05.2000) -- -----	1-12

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/FI 2003/000833**

## Box No. II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: **1-2, 4-5 and 7-12**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
**see next sheet**
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/FI 2003/000833

## Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

Present claims 1-2, 4-5 and 7-12 relate to an extremely large number of possible compounds / products. Support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds / products / claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts related to the compounds / products mentioned in claims 3 and 6 as well as for the bacteria mentioned in claim 10.

Further, the search has covered the general aspects of the invention to some extent, although it lacks the necessary precision in the definition of the subject matter. Consequently, the search for the general concept of plant-based compounds or extracts will retrieve a pertinent document only if this concept is described in general terms in a reference. Specific solutions previously known and falling under the general concept - but failing to mention this fact - are likely not to be revealed in such a search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established will not be the subject of an international preliminary examination (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/FI 2003/000833

US	2002051819	A1	02/05/2002	AU	6986601 A	02/01/2002
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				CA	2411857 A	27/12/2001
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